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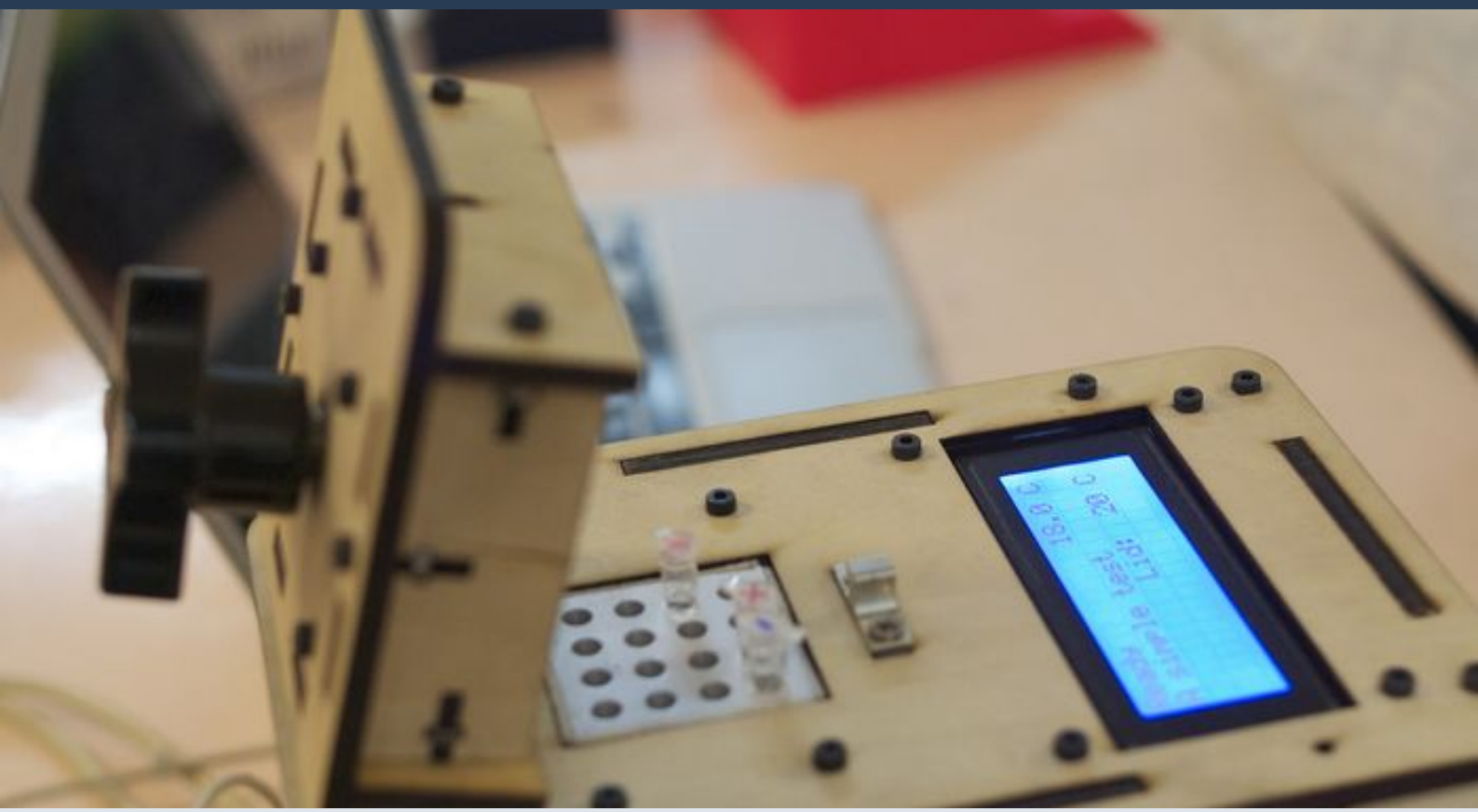
An Exquisitely Sensitive Method for Mutation Detection in Solid and Liquid Biopsies

This exquisitely sensitive method allows detection of low copy-number mutant alleles (down to 1 copy) in a mix of wild type alleles.



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IP Status

Patent application submitted

Seeking

Development partner, Commercial partner, Licensing

About **University of Nottingham**

The University of Nottingham produces world-changing research by focussing on the problems and challenges that affect societies and people on a wide scale. More than 80% of Nottingham research is ranked in the highest categories ‘world-leading’ or ‘internationally excellent’.

Background

Mutation detection is of specific interest in cancer screening, cancer surveillance, predictive testing, treatment-monitoring and tumour genotyping. Techniques for detecting mutations which are in low copy number (in a background of wild-type DNA) are expensive and/or limited in their application. This is a simple technology which can identify low copy-number mutant alleles in a range of sample types including poor quality templates (such as formalin-fixed paraffin embedded (FFPE) tissue) and in bodily fluids (such as blood, urine etc.). The high degree of specificity allows for multiplexing with high sensitivity. This opens the possibility of analysing a single sample for a range of low abundance mutations in one test. The speed and simplicity of the technique could allow liquid biopsy testing within the time frame of an outpatient appointment.

Tech Overview

- This technology consists of an adaptation of the Polymerase Chain Reaction (PCR). This exquisitely sensitive method allows detection of low copy-number mutant alleles (down to 1 copy) in a mix of wild type alleles.
- The technology is highly specific.
- The technology can be used to detect a different types of mutations including single nucleotide variants (SNV), insertion-deletion mutations (Indels), structural variants (SV).
- The technology does not require expensive equipment or consumables (such as probes). Mutation detection can be performed on a standard real-time PCR machine.
- The technology requires little or no optimisation for each test.
- The technology is quantitative over a wide dynamic range of mutant allele frequency.
- The technology is so specific it can be multiplexed to detect multiple mutations in a single sample with high sensitivity.

Figure 1: PCR amplification curve

Benefits

- Inexpensive: it employs standard PCR reagents and standard real-time PCR machines. It does not require probes. It can be detected using standard fluorescent intercalating dyes and it does not require expensive chemistries.
- Easy to interpret: the output from the assay is the presence or absence of a PCR product (i.e. yes or no signal). Easy to set up: the vast majority of the mutation-specific primers will work with standard conditions. Therefore there will be little need for optimisation.
- Adaptable for multiplexing: the specific nature of each test means that several reactions can be performed in one reaction.

- Adaptable for any mutation: if there is a priori knowledge of the mutation, then the appropriate primers can be designed whether it is a SNV, Indel or SV.
- Rapid: The assay can be completed in under 1.5 hours.
- Even when low copy-number is not an issue, the technology has other applications where specificity is required such as predictive testing in tumour tissue for drug treatment.

Applications

- The technology will transform cancer surveillance through Liquid Biopsies (detection of mutations in bodily fluids) due to (a) its exquisite sensitivity and its specificity and (b) the fact that it is simple enough to be set up in most laboratories.
- The technology can be used for cancer screening if mutation hotspots are known (e.g. KRAS mutation in pancreatic cancer).
- The quantitative nature of the technology means that it can be used to monitor responsiveness to treatment (for example through quantify mutant alleles in liquid biopsies during treatment)

Opportunity

The technique has been tested and validated. It has been used to detection mutations in the following genes; PIK3CA, BRAF, TP53, APC and KRAS. The technique has been successful in profiling DNA extracted from frozen tissue, body fluid (blood) and FFPE archived tissue. The University of Nottingham are searching for a partner to develop, commercialise or license this opportunity.

Patents

- Patent application submitted

Appendix 1

Figure 1: PCR amplification curve

A PCR amplification plot shows the enhanced sensitivity of the newly developed assay. Mutant DNA was spiked into wtDNA and was easily detectable to mutant allele frequencies as low as 0.01% (1 copy)